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I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed Andrew Gerry

Dated 29 February 2000

Patents Form 1/77

Patents Act 19
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1/77

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P01/7700 0.00 - 9903853.1

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference

PHM 99-022

2. Patent application number
(The Patent Office will fill in this part)

9903853.1

20 FEB 1999

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Zeneca Limited
15 Stanhope Gate
LONDON
W1Y 6LN, GB

Patents ADP number *(if you know it)*

6254007002

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

CHEMICAL COMPOUNDS

5. Name of your agent *(if you have one)*

TAIT, Brian Steele

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

ZENECA Pharmaceuticals
Intellectual Property Department
Mereside, Alderley Park,
Macclesfield, Cheshire, Sk10 4TG, GB

Patents ADP number *(if you know it)*

5684600002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	42 +42
Claim(s)	14
Abstract	
Drawing(s)	

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Lynda M. Slack Date 19 February 1999

12. Name and daytime telephone number of person to contact in the United Kingdom

Mrs Lynda May Slack 01625 516173

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) For details of the fee and ways to pay please contact the Patent Office.

CHEMICAL COMPOUNDS

The present invention relates to compounds that are cysteine protease inhibitors and in particular compounds that are Cathepsin L inhibitors and or Cathepsin S inhibitors especially Cathepsin S inhibitors. The invention further relates to processes for their preparation, to intermediates useful in their preparation, to their use as therapeutic agents and to pharmaceutical compositions containing them.

Cysteine proteases are enzymes important in normal cell physiology, but they are also associated with several disease states including inflammation, metastasis, tissue damage following myocardial infarction, bone resorption and muscle wasting in dystrophic diseases.

Cathepsins B, H, K, L, N and S are cysteinyl proteases involved in normal protein degradation and are normally located in the lysosomes of cells. However, when these enzymes are found outside the lysosomes they have been implicated as playing a causative role in a number of disease states including bone resorption disease such as osteoporosis.

The number of people living to an old age has increased dramatically in recent years. This has been marked by an increase in the number of people having osteoporosis and other diseases associated with old age. Osteoporosis is accompanied by a high incidence of bone fracture resulting in many aged patients being confined to their beds. There is therefore a great need for a pharmaceutical composition to treat or prevent this disease.

Living bone is continuously being remodelled and replenished by the process of resorption and deposition of the protein matrix and calcium minerals. These events are facilitated by the osteoclast, which has the ability to degrade and demineralise the bone, and the osteoblast which is responsible for new bone generation. In normal situations these processes are intimately linked resulting in little alteration of bone mass. However, pathological conditions exist in which there is an imbalance between their activity resulting in increased resorption of bone and the development of fragile and/or brittle bone structure, as seen during osteoporosis. While the exact mechanism for this resorption is not known, increased osteoclast activity, as realised by increased proteolytic activity, is a contributing factor, and selective inhibition of proteolytic action may result in the arrest or reversal of bone loss. The lysosomal cysteine proteinases, cathepsins B, H,

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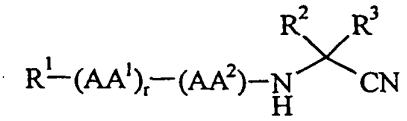
K, L, N and S have been postulated as the proteinases that are responsible for osteoclast bone resorption, because of their ability to degrade insoluble type I collagens at low pH.

Cathepsins B, H, K, L, N and S have been further implicated as playing a causative role in other diseases such as rheumatoid arthritis, osteoarthritis, tumour metastasis, pneumocystitis, Crithidia fusiculata, malaria, trypanosoma brucei brucei, schistosomiasis, periodontal disease, metachromatic leukodystrophy and muscular dystrophy.

In recent years a number of synthetic inhibitors of cysteine proteases have been disclosed. US 5,055,451 discloses a series of peptidyl methyl ketones as thiol protease inhibitors; WO 95/15749 discloses peptidyl ketones with heterocyclic leaving groups as cysteine protease inhibitors; the *in vivo* inhibition of Cathepsin B by peptidyl (acyloxy) methyl ketones was discussed in *J. Med. Chem.* **1994**, *37*, 1833-40 and these types of compounds as inhibitors of cysteine protease inhibitors were also discussed in *J. Am. Chem. Soc.*, **1988**, *110*, 4429-4431; peptidyl diazomethyl ketones as specific inactivators of thiol proteinases was discussed in *J. Biol. Chem.*, **1981**, *256*, 4, 1923-8 and in *Methods in Enzymology*, **1981**, *80*, 820-5; the inhibiting activities of 1-peptidyl-2-haloacetyl hydrazines towards Cathepsin B and calpains was discussed in *Eur. J. Med. Chem.*, **1993**, 28 297-311 and peptidyl fluoromethyl ketones as inhibitors of Cathepsin B and the implication for treatment of Rheumatoid arthritis was discussed in *Biochemical Pharmacology*, **1992**, *44*, 6, 1201-7. A review of this prior art shows that there is a great need for a specific cysteine protease and especially a Cathepsin L inhibitor and or a Cathepsin S inhibitor.

The present invention discloses compounds with inhibitory activity of cysteine proteases and in particular of Cathepsin L and or Cathepsin S.

Accordingly the present invention provides a compound of formula (I)

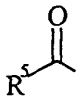


(I)

wherein:

r is 0 or 1;

R^1 optionally substituted benzyl where said optional substituents are chosen from one or more of C_{1-6}alkyl, halo, trifluoromethyl, hydroxy, trifluoromethoxy, cyano, C_{1-6}alkoxy, C_{1-6}alkanoyl, C_{1-6}alkanoyloxy, amino, C_{1-6}alkylamino, *N,N*-(C_{1-6}alkyl)$_2$amino, C_{1-6}alkanoylamino, nitro, carboxy, carbamoyl, *N*-(C_{1-6}alkyl)carbamoyl, *N,N*-(C_{1-6}alkyl)$_2$carbamoyl, C_{1-6}alkoxycarbonyl, mercapto, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, sulphamoyl, *N*-(C_{1-6}alkyl)sulphamoyl and *N,N*-(C_{1-6}alkyl)$_2$sulphamoyl or R^1 is a group of formula (II):



(II)

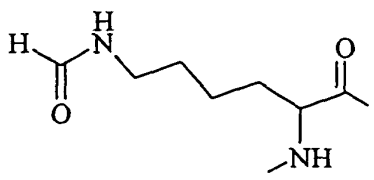
wherein R^5 is C_{1-6}alkyl (optionally substituted with an optionally substituted phenyl, an optionally substituted 5 or 6 membered heteroaryl ring, optionally substituted phenoxy or optionally substituted phenylsulphonyl), C_{1-6}alkoxy, optionally substituted phenyl, optionally substituted naphthyl, optionally substituted phenylC_{1-6}alkoxy where said optional substituents are chosen from one or more of C_{1-6}alkyl, halo, trifluoromethyl, hydroxy, trifluoromethoxy, cyano, C_{1-6}alkoxy, C_{1-6}alkanoyl, C_{1-6}alkanoyloxy, amino, C_{1-6}alkylamino, *N,N*-(C_{1-6}alkyl)$_2$amino, C_{1-6}alkanoylamino, nitro, carboxy, carbamoyl, *N*-(C_{1-6}alkyl)carbamoyl, *N,N*-(C_{1-6}alkyl)$_2$carbamoyl, C_{1-6}alkoxycarbonyl, mercapto, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, sulphamoyl, *N*-(C_{1-6}alkyl)sulphamoyl and *N,N*-(C_{1-6}alkyl)$_2$sulphamoyl;

R^2 is H, C_{1-6}alkyl [optionally substituted with one or more of hydroxy, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, R^4, R^4C_{1-6}alkylsulphanyl, R^4C_{1-6}alkylsulphinyl, R^4C_{1-6}alkylsulphonyl], or R^2 is C_{1-6}alkoxy [optionally substituted with one or more of C_{2-6}alkenyl, C_{2-6}alkynyl, R^4, R^4C_{2-6}alkenyl, R^4C_{2-6}alkynyl, Het and trifluoromethyl], or R^2 is C_{2-6}alkenyl, C_{2-6}alkynyl, C_{1-6}alkoxycarbonyl, carbamoyl, *N*-(C_{1-6}alkyl)carbamoyl, *N,N*-(C_{1-6}alkyl)$_2$carbamoyl, R^4, R^4S, R^4C_{1-6}alkylsulphanyl, *N*-(R^4C_{1-6}alkyl)carbamoyl, *N*-(HetC_{1-6}alkyl)carbamoyl, C_{1-6}alkanoylamino, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl wherein R^4 is an optionally substituted phenyl, or an optionally substituted 5 or 6 membered heteroaryl ring containing a maximum of four heteroatoms said optional substituents being chosen from one or more of C_{1-6}alkyl, halo, trifluoromethyl, hydroxy, trifluoromethoxy, cyano, C_{1-6}alkoxy,

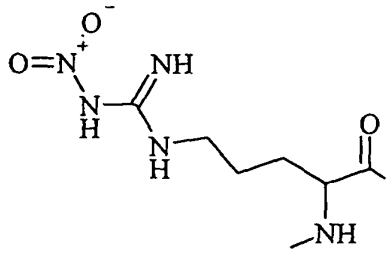
C_{1-6}alkanoyl, C_{1-6}alkanoyloxy, amino, C_{1-6}alkylamino, *N,N*-(C_{1-6}alkyl)$_2$amino, C_{1-6}alkanoylamino nitro, carboxy, carbamoyl, *N*-(C_{1-6}alkyl)carbamoyl, *N,N*-(C_{1-6}alkyl)$_2$carbamoyl, C_{1-6}alkoxycarbonyl, mercapto, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, sulphamoyl, *N*-(C_{1-6}alkyl)sulphamoyl and *N,N*-(C_{1-6}alkyl)$_2$sulphamoyl;

$\mathbf{R^3}$ is H or C_{1-6}alkyl; and

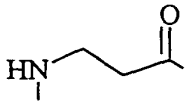
(AA^1) and **(AA^2)** are independently chosen from Ala, Arg, Cys, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Trp, Tyr, Val,



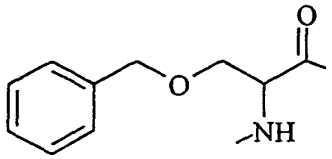
Lys(CHO),



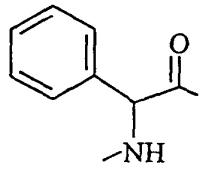
Arg(NO_2),



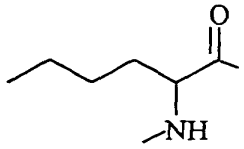
β-Ala,



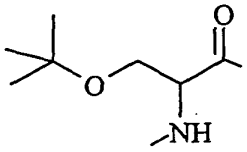
Ser(Bzl),



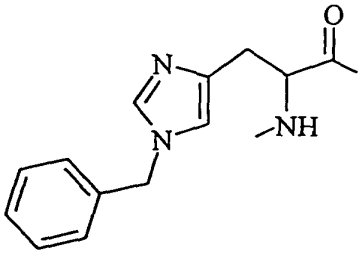
Ph-Gly,



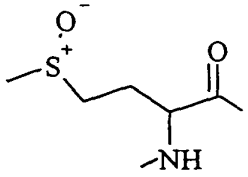
Nle,



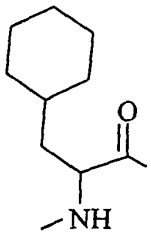
Ser(O^{t}Bu),



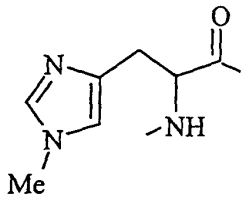
His(Bzl),



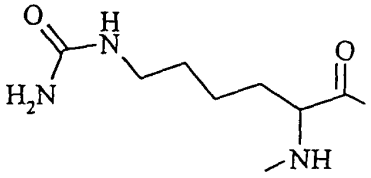
Met(O),



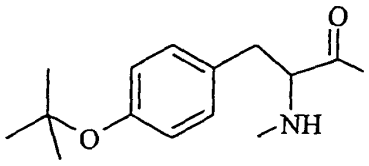
Cha,



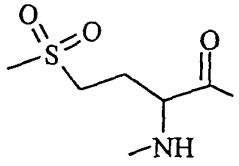
His(Me),



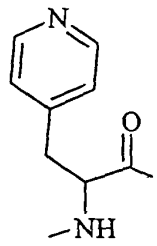
Cit,



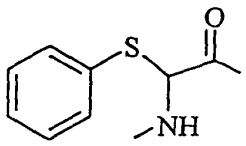
Tyr(ᵗBu),



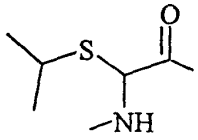
Met(O_2),



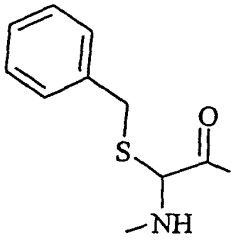
Pyr-Ala



Phe(S),



Leu(S) or



Phe(CH_2S);

wherein the nitrogen of the amino acid may optionally be alkylated with C_{1-6}alkyl and the phenyl group of Phe(S) may be optionally substituted with one or more of C_{1-6}alkyl, halo, trifluoromethyl, hydroxy, trifluoromethoxy, cyano, C_{1-6}alkoxy, C_{1-6}alkanoyl, C_{1-6}alkanoyloxy, amino, C_{1-6}alkylamino, *N,N*-(C_{1-6}alkyl)$_2$amino, C_{1-6}alkanoylamino, nitro, carboxy, carbamoyl, *N*-(C_{1-6}alkyl)carbamoyl, *N,N*-(C_{1-6}alkyl)$_2$carbamoyl, C_{1-6}alkoxycarbonyl, mercapto, C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphinyl, C_{1-6}alkylsulphonyl, sulphamoyl, *N*-(C_{1-6}alkyl)sulphamoyl and *N,N*-(C_{1-6}alkyl)$_2$sulphamoyl or the phenyl group may be fused to another phenyl group to form a naphthyl group;

or a pharmaceutically acceptable salt thereof.

In this specification the term 'alkyl' includes straight chained and branched structures and ring systems. For example, C_{1-6}alkyl includes propyl, isopropyl, *t*-butyl, cyclopropyl and

cyclohexyl. However, references to individual alkyl groups such as 'propyl' are specific for the straight chained version only, references to individual branched chain alkyl groups such as 'isopropyl' are specific for the branched chain version only and references to individual cycloalkyl groups such as cyclohexyl are specific to the cyclic groups only.

A similar convention applies to other radicals, for example "hydroxyC_{1-6}alkyl" includes 1-hydroxyethyl and 2-hydroxyethyl.

The term "halo" refers to fluoro, chloro, bromo and iodo.

"Het" means, unless otherwise further specified, a fully saturated monocyclic 5 - 8 membered heterocyclic ring, with up to 4 ring heteroatoms. Examples of "Het" include pyrrolidinyl, imidazolidinyl, pyrazolidinyl, piperidyl, piperazinyl and morpholinyl.

Unless otherwise defined standard amino acid abbreviations are used. For example "Ala" refers to alanine and "Gly" refers to glycine.

"5- or 6- membered heteroaryl ring" means, unless otherwise further specified, a 5- or 6-membered ring that contains some degree of unsaturation, with up to four ring heteroatoms selected from nitrogen, oxygen and sulphur. Examples of "5- or 6- membered heteroaryl ring" include thienyl, furyl, imidazolyl, thiazolyl, pyrimidinyl, pyridinyl, pyrrolyl and pyrazolyl.

Examples of "C_{1-6}alkanoyloxy" are acetoxy and propionyloxy. Examples of "C_{1-6}alkoxycarbonyl" include methoxycarbonyl, ethoxycarbonyl, *n*- and *t*-butoxycarbonyl. Examples of "C_{1-6}alkoxy" include methoxy, ethoxy and propoxy. Examples of "C_{1-6}alkanoylamino" include formamido, acetamido and propionylamino. Examples of "C_{1-6}alkylsulphanyl" include methylthio and ethylthio. Examples of "C_{1-6}alkylsulphinyl" include methylsulphinyl and ethylsulphinyl. Examples of "C_{1-6}alkylsulphonyl" include mesyl and ethylsulphonyl. Examples of "C_{1-6}alkanoyl" include acetyl and propionyl. Examples of "C_{1-6}alkylamino" include methylamino and ethylamino. Examples of "*N,N*-(C_{1-6}alkyl)$_2$amino" include *N,N*-dimethylamino, *N,N*-diethylamino and *N*-ethyl-*N*-methylamino. Examples of "*N*-(C_{1-6}alkyl)carbamoylC_{1-6}alkyl" are 2-(methylamino)carbonylethyl and 3-(ethylamino)carbonylpropyl. Examples of "*N,N*-(C_{1-6}alkyl)$_2$carbamoylC_{1-6}alkyl" are 2-(dimethylamino)carbonylethyl and 3-(*N*-methyl-*N*-ethylamino)carbonylpropyl. Examples of "C_{2-6}alkenyl" are vinyl, allyl and 1-propenyl. Examples of "C_{2-6}alkynyl" are ethynyl, 1-propynyl and 2-propynyl. Examples of "*N*-(C_{1-6}alkyl)carbamoyl" are *N*-methylaminocarbonyl and

N-ethylaminocarbonyl. Examples of "*N,N*-(C_{1-6}alkyl)$_2$carbamoyl" are *N,N*-dimethylaminocarbonyl and *N*-methyl-*N*-ethylaminocarbonyl. Examples of "*N*-(C_{1-6}alkyl)sulphamoyl" are *N*-methylsulphamoyl and *N*-ethylsulphamoyl. Examples of "*N,N*-(C_{1-6}alkyl)$_2$sulphamoyl" are *N,N*-dimethylsulphamoyl and *N,N*-diethylsulphamoyl. Examples of "R^4C_{1-6}alkylsulphanyl" include R^4methylthio and 2-R^4ethylthio. Examples of "R^4C_{1-6}alkylsulphinyl" include R^4methylsulphinyl and 2-R^4ethylsulphinyl. Examples of "R^4C_{1-6}alkylsulphonyl" include R^4mesyl and 2-R^4ethylsulphonyl. Examples of R^4C_{2-6}alkenyl are 2-R^4vinyl and 3-R^4allyl. Examples of "C_{2-6}alkynyl" are 2-R^4ethynyl and 3-R^4propyn-1-yl. Examples of "*N*-(R^4C_{1-6}alkyl)carbamoyl" are R^4methylaminocarbonyl and 2-R^4ethylaminocarbonyl. Examples of "*N*-(HetC_{1-6}alkyl)carbamoyl" are morpholinomethylaminocarbonyl and 2-(piperidinoethyl)aminocarbonyl.

Where optional substituents are chosen from "one or more" groups it is to be understood that this definition includes all substituents being chosen from one of the specified groups or the substituents being chosen from two or more of the specified groups. For example where optional substituents are chosen from one or more halo, C_{1-6}alkoxy and C_{1-6}alkyl, examples of possible combinations of substituents include 1) a bromo group, 2) two chloro groups, 3) a methoxy, ethoxy and propoxy substitutent, 4) a fluoro and a methoxy group, 5) a methoxy, a methyl and an ethyl group, and 6) a chloro, a methoxy and an ethyl group.

Preferred values for R^1, r, AA^1, AA^2, R^2 and R^3 are as follows.

Preferably R^1 is benzyl or a group of formula **(II)** wherein R^5 is C_{1-6}alkyl (optionally substituted with a 6 membered heteroaryl ring, phenyl, phenylsulphonyl or phenoxy optionally substituted with one or more halo), C_{1-6}alkoxy, phenyl (optionally substituted with one or more halo), naphthyl and phenylC_{1-6}alkoxy.

More preferably R^1 is benzyl or a group of formula **(II)** wherein R^5 is methyl, methoxy, ethoxy, propoxy, ^{t}butoxy, phenyl, 2,4-dichlorophenyl, naphthyl, benzyloxy, pyridylmethyl, benzyl, 2,4,6-trichlorophenoxymethyl and phenylsulphonylmethyl.

Particularly R^1 is a group of formula **(II)** wherein R^5 is methyl, ^{t}butoxy, benzyloxy and pyridylmethyl.

More particularly R^1 is a group of formula **(II)** wherein R^5 is methyl, ^{t}butoxy, benzyloxy and 4-pyridylmethyl.

In one aspect of the invention preferably r is 0.

In another aspect of the invention preferably r is 1.

Preferably AA^1 is Leu, Pyr-Ala and Phe wherein the nitrogen of the amino acid is optionally substituted with C_{1-6}alkyl.

More preferably AA^1 is Leu and the nitrogen of the amino acid is unsubstituted.

Preferably AA^2 is Phe, Leu, Ile, Tyr, Tyr(^{t}Bu), Val, Cha, Leu(S), Phe(S) and Phe(CH_2S) and the nitrogen of the amino acid is unsubstituted and the phenyl group of Phe(S) is optionally substituted with halo, C_{1-6}alkyl or is fused to another phenyl group to form a naphthyl group.

More preferably AA^2 is Tyr, Leu and Phe and the nitrogen of the amino acid is unsubstituted.

Preferred combinations of r, AA^1 and AA^2 are as follows.

When r = 0 preferably AA^2 is Phe, Leu, Ile, Val, Tyr, Tyr(^{t}Bu), Leu(S), Phe(S) and Phe(CH_2S) and the nitrogen of the amino acid is unsubstituted and the phenyl group of Phe(S) is optionally substituted with halo, C_{1-6}alkyl or is fused to another phenyl group to form a naphthyl group.

When r = 0 more preferably AA^2 is Tyr.

When r = 1 preferably AA^1-AA^2 is Leu-Leu, Pyr-Ala-Leu, Phe-Leu, Leu-Phe, Leu-Ile, Leu-Val, Leu-Cha and (N-Me)Leu-Leu.

When r = 1 more preferably AA^1-AA^2 is Leu-Leu and Leu-Phe.

Preferably R^2 is hydrogen, C_{1-6}alkyl [optionally substituted with C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphonyl or R^4], C_{1-6}alkoxy [optionally substituted with C_{2-6}alkynyl] and R^4- wherein R^4 is an optionally substituted phenyl or an optionally substituted 5 membered heteroaryl ring containing a maximum of four heteroatoms said optional substituents being chosen from one or more of C_{1-6}alkyl and halo.

More preferably R^2 is hydrogen, methyl, ethyl, propyl, isobutyl, furyl, thienyl, pyrazolyl (optionally substituted with one or more of methyl and bromo), imidazolyl, 1,2,4-triazolyl, phenyl, benzyl, 2-methylthioethyl, methylthio, ethylthio, isopropylthio, mesylethyl, methoxy, ethoxy, isopropoxy and 2-propynyloxy.

Particularly R^2 is furyl, pyrazolyl (optionally substituted with one or more of methyl and bromo), imidazolyl, 1,2,4-triazolyl, benzyl, 2-methylthioethyl, isopropylthio, methoxy, isopropoxy and 2-propynyloxy.

More particularly R^2 is fur-2-yl, pyrazol-1-yl, 3,5-dimethylpyrazol-1-yl, 4-bromo-3,5-dimethylpyrazol-1-yl, imidazol-1-yl, 1,2,4-triazol-1-yl, benzyl, methylthioethyl, isopropylthio, methoxy, isopropoxy and 2-propynyloxy.

Preferably R^3 is hydrogen.

According to another aspect of the present invention there is provided a compound of the formula **(I)** wherein:

R^1 is benzyl or a group of formula **(II)** wherein R^5 is C_{1-6}alkyl (optionally substituted with a 6 membered heteroaryl ring, phenyl, phenylsulphonyl or phenoxy optionally substituted with one or more halo), C_{1-6}alkoxy, phenyl (optionally substituted with one or more halo), naphthyl or phenylC_{1-6}alkoxy;

r is 0 or 1;

AA^1 is Leu, Pyr-Ala or Phe wherein the nitrogen of the amino acid is optionally substituted with C_{1-6}alkyl;

AA^2 is Phe, Leu, Ile, Tyr, Tyr(^{t}Bu), Val, Cha, Leu(S), Phe(S) and Phe(CH_2S) and the nitrogen of the amino acid is unsubstituted and the phenyl group of Phe(S) is optionally substituted with halo, C_{1-6}alkyl or is fused to another phenyl group to form a naphthyl group;

R^2 is hydrogen, C_{1-6}alkyl [optionally substituted with C_{1-6}alkylsulphanyl, C_{1-6}alkylsulphonyl or R^4], C_{1-6}alkoxy [optionally substituted with C_{2-6}alkynyl,] or R^4- wherein R^4 is an optionally substituted phenyl or an optionally substituted 5 membered heteroaryl ring containing a maximum of four heteroatoms said optional substituents being chosen from one or more of C_{1-6}alkyl or halo; and

R^3 is hydrogen;

or a pharmaceutically acceptable salt thereof.

A further preferred class of compounds is that of formula **(I)** wherein:

R^1 is a group of formula **(II)** wherein R^5 is methyl, ^{t}butoxy, benzyloxy or pyridylmethyl;

r is 0 or 1;

AA^1 is Leu wherein the nitrogen of the amino acid is unsubstituted;

AA^2 is Tyr, Leu or Phe wherein the nitrogen of the amino acid is unsubstituted;

R^2 is furyl, pyrazolyl (optionally substituted with one or more methyl or bromo), imidazolyl, 1,2,4-triazolyl, benzyl, methylthioethyl, isopropylthio, methoxy, isopropoxy or propynyloxy; and

R^3 is hydrogen;

or a pharmaceutically acceptable salt thereof.

Preferred compounds are those of Examples 1 - 58 or a pharmaceutically acceptable salt thereof.

Especially preferred compounds are those of Examples 8, 13, 15, 17, 19, 25, 30, 31, 32, 33, 34, 35, 36, 37, 38, 40 and 41 or a pharmaceutically acceptable salt thereof.

Suitable pharmaceutically acceptable salts include acid addition salts such as the methanesulphonate, fumarate, hydrochloride, hydrobromide, citrate and maleate salts and salts formed with phosphoric and sulphuric acid. In another aspect suitable salts are base salts such as an alkali metal salt for example a sodium salt, an alkaline earth metal salt for example a calcium or a magnesium salt, an organic amine salt for example a salt with triethylamine, morpholine, *N*-methylpiperidine, *N*-ethylpiperidine, procaine, dibenzylamine, *N,N*-dibenzylethylamine or an amino acid for example a lysine salt. There may be more than one cation or anion depending on the number of charged functions and the valency of the cations or anions. A preferred pharmaceutically acceptable salt is a sodium salt.

Some compounds of formula (I) may possess chiral centres. It is to be understood that the invention encompasses all such optical isomers and diasteroisomers of compounds of formula (I) which possess cysteine protease inhibitory activity.

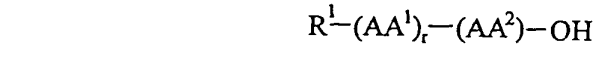
The invention further relates to all tautomeric forms of the compounds of formula (I).

It is also to be understood that certain compounds of the formula (I) can exist in solvated as well as unsolvated forms such as, for example, hydrated forms. It is to be understood that the invention encompasses all such solvated forms.

Another aspect of the present invention provides a process for preparing a compound of formula (I) or a pharmaceutically acceptable salt thereof. According to this aspect of the

invention there is provided a process (in which variable groups are as defined for formula (I) unless otherwise stated) which comprises:

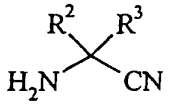
a) coupling an acid of formula **(III)**:



(III)

or a reactive derivative thereof;

with an amine of formula **(IV)**:



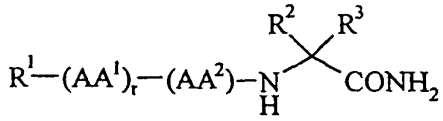
(IV)

A suitable reactive derivative of an acid of the formula **(III)** is, for example, an acyl halide, for example an acyl chloride formed by the reaction of the acid and an inorganic acid chloride, for example thionyl chloride; a mixed anhydride, for example an anhydride formed by the reaction of the acid and a chloroformate such as isobutyl chloroformate; an active ester, for example an ester formed by the reaction of the acid and a phenol such as pentafluorophenol, an ester such as pentafluorophenyl trifluoroacetate, an alcohol such as 1-hydroxybenzotriazole or a uronium salt such as 2-(1-benzotriazolyl)-1,1,3,3-tetramethyluronium hexafluorophosphate(V); an acyl azide, for example an azide formed by the reaction of the acid and an azide such as diphenylphosphoryl azide; an acyl cyanide, for example a cyanide formed by the reaction of an acid and a cyanide such as diethylphosphoryl cyanide; or the product of the reaction of the acid and a carbodiimide such as *N,N*-dicyclohexylcarbodiimide or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide.

The reaction is preferably carried out in the presence of a suitable base such as, for example, an alkali or alkaline earth metal carbonate, alkoxide or hydroxide, for example sodium carbonate or potassium carbonate, or, for example, an organic amine base such as, for example, pyridine, 2,6-lutidine, collidine, 4-dimethylaminopyridine, triethylamine, morpholine or diazabicyclo-[5.4.0]undec-7-ene. The reaction is also preferably carried out in a suitable inert solvent or diluent, for example methylene chloride, acetonitrile, tetrahydrofuran, 1,2-

dimethoxyethane, *N,N*-dimethylformamide, *N,N*-dimethylacetamide, *N*-methylpyrrolidin-2-one or dimethylsulphoxide, and at a temperature in the range, for example, -78° to 150°C, conveniently at or near ambient temperature.

b) dehydrating a compound of formula **(V)**:

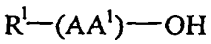


(V)

under standard conditions.

For example such a dehydration reaction may conventionally be carried out by reaction with a reagent such as trifluoroacetic anhydride. The reaction can conveniently be conducted in the presence of a suitable base as defined hereinbefore such as, for example, triethylamine. The reaction is also preferably carried out in a suitable inert solvent or diluent, as defined hereinbefore such as dichloromethane and at a temperature in the range, for example, -10°C to reflux conveniently 10°C to reflux.

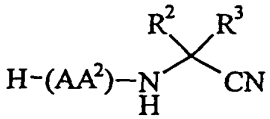
c) for compounds of formula **(I)** where r = 1, coupling an acid of formula **(VI)**:



(VI)

or a reactive derivative thereof as defined hereinbefore;

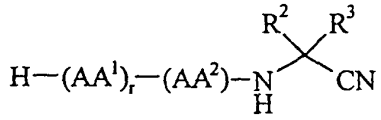
with an amine of formula **(VII)**:



(VII)

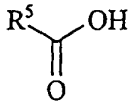
The reaction can conveniently be conducted under standard coupling conditions, such as those described in a) above.

d) For compounds of formula (I) where R^1 is a group of formula (II) reaction of an amine of formula (VIII):



(VIII)

with an acid of formula (IX):

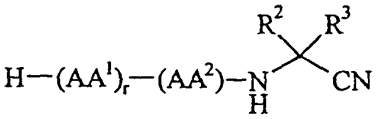


(IX)

or a reactive derivative thereof as defined hereinbefore.

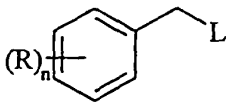
The reaction can conveniently be conducted under standard coupling conditions, such as those described in a) above.

e) Compounds of formula (I) where R^1 is optionally substituted benzyl may be obtained by reaction of an amine of formula (X):



(X)

i) with a compound of formula (XI):



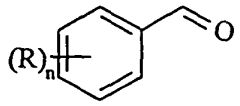
(XI)

where $(R)_n$ are optional substituents as defined above and L is a displaceable group.

A suitable displaceable group L is, for example, a halogeno or sulphonyloxy group, for example a chloro, bromo, methanesulphonyloxy or toluene-4-sulphonyloxy group.

This reaction may be carried out under standard conditions such as, for example, those described in *Synthesis* **1993**, *12*, 1243-6; or

ii) by reaction with an aldehyde of formula **(XII)**:



(XII)

This reaction may be carried out under standard conditions such as, for example, those described in *Synth. Commun.*, **1995**, *25*, *18*, 2819-2827.

If not commercially available, the necessary starting materials for the procedures described above may be made by procedures which are selected from standard organic chemical techniques, techniques which are analogous to the synthesis of known, structurally similar compounds, by techniques which are analogous to the above described procedures or by techniques which are analogous to the procedures described in the examples.

For example, it will be appreciated that certain of the optional substituents on a phenyl or naphthyl or a heteroaryl ring in the compounds of the present invention may be introduced by standard aromatic substitution reactions or generated by conventional functional group modifications either prior to or immediately following the processes mentioned above, and as such are included in the process aspect of the invention. Such reactions and modifications include, for example, introduction of a substituent by means of an aromatic substitution reaction, reduction of substituents, alkylation of substituents and oxidation of substituents. The reagents and reaction conditions for such procedures are well known in the chemical art. Particular examples of aromatic substitution reactions include the introduction of a nitro group using concentrated nitric acid, the introduction of an acyl group using, for example, an acyl halide and a Lewis acid (such as aluminium trichloride) under Friedel Crafts conditions; the introduction of an alkyl group using an alkyl halide and Lewis acid (such as aluminium trichloride) under Friedel Crafts conditions; and the introduction of a halogeno group. Particular examples of modifications include the reduction of a nitro group to an amino group by, for example, catalytic hydrogenation with a nickel catalyst or treatment with iron in the presence of hydrochloric acid with heating; oxidation of alkylthio to alkylsulphinyl or alkylsulphonyl.

It will also be appreciated that in some of the reactions mentioned herein it may be necessary/desirable to protect any sensitive groups in the compounds. The instances where protection is necessary or desirable and suitable methods for protection are known to those skilled in the art. Thus, if reactants include groups such as amino, carboxy or hydroxy it may be desirable to protect the group in some of the reactions mentioned herein.

A suitable protecting group for an amino or alkylamino group is, for example, an acyl group, for example an alkanoyl group such as acetyl, an alkoxycarbonyl group, for example a methoxycarbonyl, ethoxycarbonyl or *t*-butoxycarbonyl group, an arylmethoxycarbonyl group, for example benzyloxycarbonyl, or an aroyl group, for example benzoyl. The deprotection conditions for the above protecting groups necessarily vary with the choice of protecting group. Thus, for example, an acyl group such as an alkanoyl or alkoxycarbonyl group or an aroyl group may be removed for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide. Alternatively an acyl group such as a *t*-butoxycarbonyl group may be removed, for example, by treatment with a suitable acid as hydrochloric, sulphuric or phosphoric acid or trifluoroacetic acid and an arylmethoxycarbonyl group such as a benzyloxycarbonyl group may be removed, for example, by hydrogenation over a catalyst such as palladium-on-carbon, or by treatment with a Lewis acid for example boron tris(trifluoroacetate). A suitable alternative protecting group for a primary amino group is, for example, a phthaloyl group which may be removed by treatment with an alkylamine, for example dimethylaminopropylamine, or with hydrazine.

A suitable protecting group for a hydroxy group is, for example, an acyl group, for example an alkanoyl group such as acetyl, an aroyl group, for example benzoyl, or an arylmethyl group, for example benzyl. The deprotection conditions for the above protecting groups will necessarily vary with the choice of protecting group. Thus, for example, an acyl group such as an alkanoyl or an aroyl group may be removed, for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide. Alternatively an arylmethyl group such as a benzyl group may be removed, for example, by hydrogenation over a catalyst such as palladium-on-carbon.

A suitable protecting group for a carboxy group is, for example, an esterifying group, for example a methyl or an ethyl group which may be removed, for example, by hydrolysis with a

base such as sodium hydroxide, or for example a *t*-butyl group which may be removed, for example, by treatment with an acid, for example an organic acid such as trifluoroacetic acid, or for example a benzyl group which may be removed, for example, by hydrogenation over a catalyst such as palladium-on-carbon.

The protecting groups may be removed at any convenient stage in the synthesis using conventional techniques well known in the chemical art.

Many of the intermediates defined herein are novel, for example, those of the formula (V) and these are provided as a further feature of the invention. Moreover some of the starting materials for use in process variant (b) described hereinbefore, namely those compounds of the formula **(VIII)** are not only novel but also active as inhibitors of Cathepsin L and or Cathepsin S. Accordingly these compounds are provided as a further feature of the invention.

According to a further feature of the invention there is provided a compound of the formula **(I)**, or a pharmaceutically acceptable salt thereof, for use in a method of treatment of the human or animal body by therapy.

According to a further feature of the present invention there is provided a method for producing inhibition of a cysteine protease in a warm blooded animal, such as man, in need of such treatment, which comprises administering to said animal an effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof.

The invention also provides a compound of the formula **(I)**, or a pharmaceutically acceptable salt thereof, for use as a medicament; and the use of a compound of the formula **(I)** of the present invention, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in the inhibition of a cysteine protease in a warm blooded animal, such as man.

In particular the invention provides the use of a compound of the formula **(I)** of the present invention, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in the inhibition of Cathepsin S in a warm blooded animal, such as man.

In order to use a compound of the formula **(I)** or a pharmaceutically acceptable salt thereof for the therapeutic treatment of mammals including humans, in particular in the inhibition of a cysteine protease, it is normally formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition.

Therefore in another aspect the present invention provides a pharmaceutical composition which comprises a compound of the formula (I) or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable diluent or carrier.

The pharmaceutical compositions of this invention may be administered in standard manner for the disease condition that it is desired to treat, for example by oral, rectal or parenteral administration. For these purposes the compounds of this invention may be formulated by means known in the art into the form of, for example, tablets, capsules, aqueous or oily solutions or suspensions, (lipid) emulsions, dispersible powders, suppositories, ointments, creams, drops and sterile injectable aqueous or oily solutions or suspensions.

A suitable pharmaceutical composition of this invention is one suitable for oral administration in unit dosage form, for example a tablet or capsule which contains between 100 mg and 1 g of the compound of this invention.

In another aspect a pharmaceutical composition of the invention is one suitable for intravenous, subcutaneous or intramuscular injection.

Each patient may receive, for example, an intravenous, subcutaneous or intramuscular dose of 1 $mgkg^{-1}$ to 100 $mgkg^{-1}$ of the compound, preferably in the range of 5 $mgkg^{-1}$ to 20 $mgkg^{-1}$ of this invention, the composition being administered 1 to 4 times per day. The intravenous, subcutaneous and intramuscular dose may be given by means of a bolus injection. Alternatively the intravenous dose may be given by continuous infusion over a period of time. Alternatively each patient will receive a daily oral dose which is approximately equivalent to the daily parenteral dose, the composition being administered 1 to 4 times per day.

The following illustrate representative pharmaceutical dosage forms containing the compound of formula (I), or a pharmaceutically-acceptable salt thereof (hereafter compound X), for therapeutic or prophylactic use in humans:

Tablet I	mg/tablet
Compound X.	100
Lactose Ph.Eur.	179
Croscarmellose sodium	12.0
Polyvinylpyrrolidone	6
Magnesium stearate	3.0

(b)

Tablet II	mg/tablet
Compound X	50
Lactose Ph.Eur.	229
Croscarmellose sodium	12.0
Polyvinylpyrrolidone	6
Magnesium stearate	3.0

(c)

Tablet III	mg/tablet
Compound X	1.0
Lactose Ph.Eur.	92
Croscarmellose sodium	4.0
Polyvinylpyrrolidone	2.0
Magnesium stearate	1.0

(d)

Capsule	mg/capsule
Compound X	10
Lactose Ph.Eur.	389

Croscarmellose sodium	100
Magnesium stearate	1.

(e)

Injection I	(50 mg/ml)
Compound X	5.0% w/v
Isotonic aqueous solution	to 100%

Buffers, pharmaceutically-acceptable cosolvents such as polyethylene glycol, polypropylene glycol, glycerol or ethanol or complexing agents such as hydroxy-propyl β cyclodextrin may be used to aid formulation.

Note

The above formulations may be obtained by conventional procedures well known in the pharmaceutical art. The tablets (a)-(c) may be enteric coated by conventional means, for example to provide a coating of cellulose acetate phthalate.

Inhibition of Cathepsin L and S.

The pharmaceutically-acceptable compounds of the present invention are useful in the inhibition of cathepsin L and cathepsin S, having a good activity *in vitro* against human cathepsin L, human cathepsin S and rabbit Cathepsin L.

Cathepsin L Assay

Recombinant human Cathepsin L was cloned and expressed in E Coli and purified using the method as described by Zeneca Limited, GB 2 306 961 A (published 14.05.1997).

Rabbit cathepsin L was purified from rabbit liver as described by Maciewicz R. A. and Etherington D. J. (Biochem. J. (1988) 256, 433-440) except the liver homogenate supernatant was concentrated by fractionation with $(NH_4)_2SO_4$ (20-80% saturation), and the pellet taken up and dialysed against 20mM NaAcetate pH 5.5, 1mM ethylenediaminetetraacetic acid (EDTA). The supernatant was then applied to a CM Sepharose ion exchange column and cathepsin L eluted by

gradient elution (0.25-0.75M NaCl). Fraction activity was determined using the synthetic substrate NCBz-Phe-Arg-NHMec as described. Cathepsin L fractions were pooled and desalted on a Sephacryl S100 column. Active fractions were pooled, adjusted to 20% saturation $(NH_4)_2SO_4$ and concentrated on a phenyl sepharose column. The remaining purification steps were as described.

Cathepsin L activity was measured based on the method of Barrett and Kirschke (1981 Methods in Enzymology, **80**, 535-561), using the fluorogenic substrates NCBz-Phe-Arg-NHMec. Inhibitors were identified by their ability to decrease the generation of the fluorescent leaving group (NHMec). Briefly the assay was as follows:

rHuman cathepsin L or rabbit cathepsin L (0.025 pmoles) was pre-incubated with or without test compound in 0.1M sodium acetate buffer pH4.5, 10mM cysteine, 0.1% Brij 35 at 25° C for 15 minutes in a solid black 96 well plate. Synthetic substrate, 20μM NCBz-Phe-Arg-NHMec, was added and the mixture incubated at 37°C for 30 minutes. The reaction was stopped by the addition of 0.1M sodium chloroacetate pH 4.3. Fluorescence was determined using a Fluoroskan II plate reader; excitation 355nm, emission 460nm. Compound potency was determined from the raw fluorescence data by calculating the IC_{50} against each enzyme using a PC graph drawing software package.

Cathepsin S assay.

Cloning and Expression of human cathepsin S.

Recombinant human cathepsin S was cloned and expressed in Baculovirus, by the following method. The cDNA sequence for human cathepsin S is available in the EMBL database Accession Number M90696. This database sequence was used to prepare, by PCR on mRNA from human tissues, a recombinant plasmid carrying an insert with a DNA sequence identical to that of cathepsin S in the EMBL database (Acc No M90696). The techniques for mRNA isolation, PCR and cloning are standard techniques known by those skilled in the art. Sequence determination of the recombinant insert was carried out using established DNA sequencing techniques.

The PCR was done so as to introduce an EcoRI cloning site 5' of the 'ATG' of cathepsin S and an XbaI cloning site 3' of the 'Stop' codon. The PCR product was cloned between the

EcoRI and XbaI sites of the baculovirus transfer vector pFASTBAC-1 (Bac-to-Bac Expression System commercially available from Gibco BRL –Life Technologies (cat no 10359-016)). This recombinant construct was used to generate, by standard techniques, a recombinant baculovirus capable of expressing preprocathepsin S.

Expression of recombinant cathepsin S was tested for the baculoviral constructs by infection of two insect cell lines : Sf9 cells (ATCC No CRL-1711) and T.ni cells (Invitrogen, Cat No B855-02).

Purification of cathepsin S

Method 1.

Procathepsin S was found in the insect cell medium and acid activated. The medium was mixed with an equal volume of 100mM Sodium Acetate buffer pH 4.5, 5mM dithiothreitol (DTT) and 5mM EDTA and incubated for one hour at 37°C method of Maubach et al (Eur. J. Biochem., 250, 745-750, 1997).

Method 2.

The pH of insect cell medium (10ml) containing procathepsin S was adjusted to 4.5 with glacial acetic acid and DTT and EDTA added to 5mM. The sample was then incubated at 37°C for 150min to enable conversion to the active enzyme. Ammonium sulphate was then added to 80% saturation and a pellet obtained by centrifugation. This pellet was redissolved in 2ml buffer A (100mM Tris, 500mM NaCl, 1mM EDTA, pH7.5) and mixed in a batchwise fashion with 100µl thiopropyl-Sepharose for 15min at 4°C. The non bound fraction was removed by a brief centrifugation and the gel washed with 2x1ml buffer A. Cathepsin S was then eluted by batch mixing with 0.4ml 20mM DTT in buffer A for 15min at 4°C.

Measurement of cathepsin S Activity.

Cathepsin S activity was measured based on the method of Maubach et al (Eur. J. Biochem., 250, 745-750, 1997), using the fluorogenic substrate Z-Val-Val-Arg-NHMec. Inhibitors were identified by their ability to decrease the generation of the fluorescent leaving group (NHMec). Briefly the assay was as follows:

rHuman cathepsin S (1.5 nmoles) was pre-incubated with or without compounds in 50m Potassium phosphate buffer pH 6.0-6.2, 20mM Na_2EDTA, 0.1% Brij at 25°C for 5 minutes in a solid black 96 well plate. Synthetic substrate, 20μM Z-Val-Val-Arg-NHMec, was added and the mixture incubated at 30°C for 20 minutes. The reaction was stopped by the addition of 0.1M sodium chloroacetate pH 4.3. Fluorescence was determined using a Fluoroskan II plate reader; excitation 355nm, emission 460nm. Compound potency was determined from the raw fluorescence data by calculating the IC_{50} against cathepsin S using a PC graph drawing software package.

The following results were obtained on a standard *in-vitro* test system for the inhibition of Cathepsin L. The activity is described in terms of IC_{50}.

When tested in the above *in-vitro* tests the compounds of this invention give IC_{50}s in the range 1-10,000 nM.

The following data was obtained for Examples 1, 19 and 26:

Example	IC_{50} (Human) (nM)	IC_{50} (Rabbit) (nM)
1		297
19	38	38.27
26	5651	

The invention will now be illustrated by the following non-limiting examples in which, unless stated otherwise:

(i) temperatures are given in degrees Celsius (°C); operations were carried out at room or ambient temperature, that is, at a temperature in the range of 18-25°C;

(ii) organic solutions were dried over anhydrous magnesium sulphate; evaporation of solvent was carried out using a rotary evaporator under reduced pressure (600-4000 Pascals; 4.5-30 mm Hg) with a bath temperature of up to 60°C;

(iii) chromatography means flash chromatography on silica gel; thin layer chromatography (TLC) was carried out on silica gel plates;

(iv) in general, the course of reactions was followed by TLC and reaction times are given for illustration only;

(v) final products had satisfactory proton nuclear magnetic resonance (NMR) spectra;

(vi) yields are given for illustration only and are not necessarily those which can be obtained by diligent process development; preparations were repeated if more material was required;

(vii) when given, NMR data is in the form of delta values for major diagnostic protons, given in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard, determined at 250 MHz using perdeuterio dimethyl sulphoxide (DMSO-δ_6) as the solvent unless otherwise stated;

(viii) chemical symbols have their usual meanings; SI units and symbols are used;

(ix) solvent ratios are given in percentage by volume;

(x) mass spectra (MS) were run with an electron energy of 70 electron volts in the chemical ionisation (CI) mode using a direct exposure probe; where indicated ionisation was effected by electron impact (EI) or fast atom bombardment (FAB); where values for m/z are given, generally only ions which indicate the parent mass are reported;

(xi) melting points are uncorrected and (dec) indicates decomposition; the melting points given are those obtained for the materials prepared as described; polymorphism may result in isolation of materials with different melting points in some preparations; and

(xii) Z refers to benzyloxycarbonyl and Boc refers to *tert*-butoxycarbonyl.

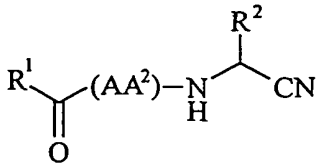
Example 1

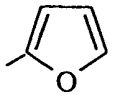
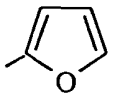
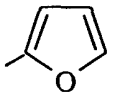
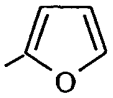
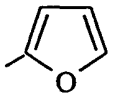
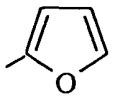
2-(Boc-L-phenylalanyl)-2-(2-furyl)acetonitrile

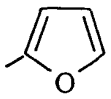
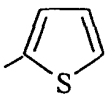
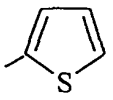
A mixture of Boc-L-phenylalanine (5.0 g), 2-(2-furyl)acetonitrile (3.0 g), hydroxybenzotriazole (5.1 g), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (4.0 g) and triethylamine (2.63 ml) in *N,N*-dimethylformamide (950 ml) was stirred at 0°C for 30 minutes and then at ambient temperature for 14 hours. The solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate (100 ml) and water (100 ml). The ethyl acetate layer was separated and washed with 2M hydrochloric acid, brine, saturated aqueous sodium bicarbonate and dried. The residue obtained on removal of the solvent was subjected to chromatography on silica by elution with a mixture of dichloromethane and ethyl acetate (9:1

v/v) to give 2-(Boc-L-phenylalanyl)-2-(2-furyl)acetonitrile (4.53 g). Mp 157-158°C; m/z 370 $(MH)^+$; NMR ($CDCl_3$) 1.4 (s, 9H), 3.1 (m, 2H), 4.37 (q, 1H), 4.87 (m, 1H), 6.1 (d, 1H), 6.39 (m, 1H), 6.45 (d, 1H), 6.72 (m, 1H), 7.22 (m, 5H), 7.41 (d, 1H).

Using this method but with appropriate starting materials there were prepared the following compounds:



Example	R^1	AA^2	R^2	MH^+	Mp (°C)
2	^{t}BuO-	Leu		336	
3	Me	Leu			152-4
4	Me	Leu			95-8
5	^{t}BuO-	Ile			106-8
6	^{t}BuO-	Val			106-9
7	^{t}BuO-	Leu	H		110-2
8	$PhCH_2O$-	Tyr		420	

9	$PhCH_2O$-	Tyr(^{t}Bu)			97-100
10	$PhCH_2O$-	Tyr			140
11	$PhCH_2O$-	Phe			131-2
12	^{t}BuO-	Leu	Ph	346	120-121

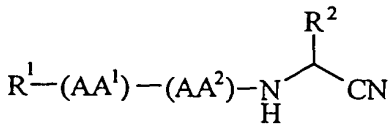
Example 13

(2S)-2-(Z-Leu-Leu-NH)-3-phenylpropionitrile

Trifluoroacetic anhydride (0.28 ml) was added dropwise to a mixture of Z-Leu-Leu-Phe-NH_2 (0.9 g) and pyridine (10 ml) which was stirred under argon at -10°C. The mixture was allowed to warm to room temperature over 1 hour, diluted with water and extracted with ethyl acetate. The extract was washed successively with 1M hydrochloric acid and brine, dried and evaporated to dryness and the residue was recrystallized from ethyl acetate/hexane to give (2S)-2-(Z-Leu-Leu-NH)-3-phenylpropionitrile (0.57 g). Mp 152-154°C; m/z 507 $(MH)^+$; NMR 0.75-0.95 (m, 12H), 1.3-1.7 (m, 6H), 3.05 (d, 2H), 4.05 (m, 1H), 4.3 (m, 1H), 4.9 (m, 1H), 7.2-7.45 (m, 11H), 7.85 (d, 1H), 8.75 (d, 1H).

Example 14-19:

The following compounds were prepared by a similar process to that described in Example 13:



Example	R^1	AA^1	AA^2	R^2	Mp (°C)	MH^+
14 [1,2]	BOC	Leu	Leu	$MeSCH_2CH_2$-	129-130	457
15 [1,2]	Z	Leu	Leu	$MeSCH_2CH_2$-	-	491
16 [1,2]	PhCO	Pyr-Ala	Leu	$MeSCH_2CH_2$-	-	496
17 [1,3]	Z	Leu	Leu	i-PrS-	-	491
18 [1,3]	Z	Phe	Leu	i-PrS-	-	525
19 [1,3]	Z	Leu	Phe	i-PrS-	163-165	525

Example 20

(2S)-2-(Z-Phe-Leu-NH)-4-methylthiobutyronitrile

Phosphoryl chloride (0.07 ml) was added dropwise to stirred, ice-cooled *N,N*-dimethylformamide (2 ml) and the resulting solution added to a stirred ice cooled solution of Z-Phe-Leu-Met-NH_2 (163 mg) in *N,N*-dimethylformamide (2 ml) under an atmosphere of argon. The mixture was stirred for 0.5 hours then poured into ice-water and extracted with ethyl acetate. The extract was washed with water, dried and evaporated to dryness. The residue was recrystallized from ethyl acetate and hexane to give (2S)-2-(Z-Phe-Leu-NH)-4-methylthiobutyronitrile (64 mg). Mp140-144°C; m/z 525 $(MH)^+$; NMR 0.7-0.95 (m, 6H), 1.1-1.5 (m, 3H), 2.0-2.2 (m, 5H), 2.45-2.65(m, 2H), 2.7-3.0 (m, 2H), 4.25 (m, 2H), 4.65 (q, 1H), 5.0 (m, 2H), 7.1-7.45 (m, 10H), 7.7 (d, 1H), 8.3 (d, 1H), 8.5 (d, 1H).

Example 21

(2S)-2-[(N-phenylacetyl-N-methyl-Leu)-Leu-NH]-4-methylthiobutyronitrile

The process described in Example 20 was repeated using (2S)-2-[(N-phenylacetyl-N-methyl-Leu)-Leu-Met-NH_2 instead of Z-Phe-Leu-Met-NH_2 as starting material to give (2S)-2-[(N-phenylacetyl-N-methyl-Leu)-Leu-NH]-4-methylthiobutyronitrile. M/z 489 $(MH)^+$; NMR [at 100

---

[1] Purified by flash chromatography on silica (Merck, ART 9385) using mixtures of ethyl acetate and hexane as eluent.

[2] S isomer at aminoacetonitrile.

[3] mixture of epimers at aminoacetonitrile

°C] 0.9 (m, 12H), 1.4-1.8 (m, 6H), 2.0-2.2 (m, 5H), 2.55 (m, 2H), 2.95 (m, 3H), 3.75 (s, 2H), 4.35 (m, 1H), 4.75-5.0 (m, 2H), 7.1-7.5 (m, 6H), 8.25 (m, 1H).

Example 22

(2S)-2-[(N-benzoyl-N-methyl-Leu)-Leu-NH]-4-methylthiobutyronitrile

The process described in Example 20 was repeated using (2S)-2-[(N-benzoyl-N-methyl-Leu)-Leu-Met-NH_2 instead of Z-Phe-Leu-Met-NH_2 as starting material to give (2S)-2-[(N-benzoyl-N-methyl-Leu)-Leu-NH]-4-methylthiobutyronitrile. M/z 475 $(MH)^+$; NMR [at 100 °C] 0.9 (m, 12H), 1.5-1.8 (m, 6H), 2.0-2.2 (m, 5H), 2.55 (m, 2H), 2.9 (m, 3H), 4.4 (m, 1H), 4.75-5.0 (m, 2H), 7.3-7.6 (m, 6H), 8.3 (m, 1H).

Example 23

(2S)-2-(Z-Leu-Leu-NH)-4-methylsulphonylbutyronitrile

A mixture of (2S)-2-(Z-Leu-Leu-NH)-4-methylthiobutyronitrile (80 mg), oxone (150 mg), ethanol (2 ml) and water (1 ml) was stirred at room temperature for 18 hours. The mixture was diluted with water and extracted with ethyl acetate. The extract was dried and evaporated to dryness. The residue was purified by flash chromatography on silica (Merck, ART 9385) using increasingly polar mixtures of ethyl acetate and hexane as eluent to give (2S)-2-(Z-Leu-Leu-NH)-4-methylsulphonylbutyronitrile (80 mg). M/z 489 $(MH)^+$; NMR ($CDCl_3$) 0.8-1.0 (m, 12H), 1.3-1.6 (m, 15H), 2.4 (m, 2H), 3.0 (s, 3H), 3.2 (m, 2H), 4.05 (m, 1H), 4.35 (m, 1H), 4.7-5.05 (m, 2H), 6.55 (d, 1H), 7.7 (d, 1H).

Example 24

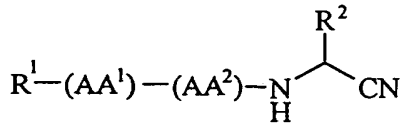
(2RS)-2-(Z-Leu-Leu-NH)-2-phenylacetonitrile

A mixture of (2RS)-2-(BOC-LeuNH)-2-phenylacetonitrile (0.69 g), dichloromethane (10 ml) and *N,N*-diisopropylethylamine (0.5 ml) was stirred under an argon atmosphere and iodotrimethylsilane (0.36 g) was added dropwise. The mixture was stirred for 1 hour and then additional iodotrimethylsilane (0.36 g) was added and the mixture was stirred for a further hour. N-methylmorpholine (0.5 ml) was added followed by methanol (0.5 ml) and then the solution was evaporated to dryness.

A mixture of the residue, *N,N*-dimethylformamide (10 ml), Z-LeuOH (0.58 g), 1-hydroxybenzotriazole (0.3 g), 1-dimethylaminopropyl-3-ethylcarbodiimide (0.42 g) and N-methylmorpholine (1 ml) was stirred at room temperature for 18hours.The mixture was evaporated to dryness and the residue dissolved in ethyl acetate, and the solution was washed successively with 0.5M hydrochloric acid, 1M sodium hydroxide and brine and then dried and evaporated to dryness. The residue was purified by flash chromatography on silica (Merck, ART 9385) using increasingly polar mixtures of ethyl acetate and hexane as eluent to give (2RS)-2-(Z-Leu-Leu-NH)-phenylacetonitrile (0.45 g). Mp 168-170°C; m/z 493 $(MH)^+$; NMR 0.75-0.95 (m, 12H), 1.25-1.75 (m, 6H), 4.05 (m, 1H), 4.4 (m, 1H), 5.0 (m, 2H), 6.15 (m, 1H), 7.3-7.5 (m, 11H), 7.95 (m, 1H), 9.15-9.3 (m, 1H).

Examples 25-29:

The following compounds were prepared by a similar process to that described in Example 24:



Example	R^1	AA^1	AA^2	R^2	MH^+
25	Boc	Leu	Leu	furan-2-yl	449
26	Boc	Leu	Phe	furan-2-yl	483
27	Boc	Leu	Ile	furan-2-yl	449
28	Boc	Leu	Val	furan-2-yl	435
29	Z	Leu	Leu	H	417

Example 30

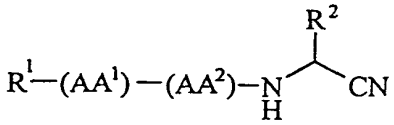
(2RS)-2-(Z-Leu-Leu-NH)-2-methoxyacetonitrile

N-bromosuccinimide (133 mg) was added to a stirred solution of (2RS)-2-(Z-Leu-Leu-NH)-2-(2-propylthio)acetonitrile (245 mg) in methanol (10 ml), the mixture was allowed to warm

to room temperature and it was then stirred at room temperature for 1 hour. The mixture was evaporated to dryness and the residue was partitioned between ethyl acetate and water. The ethyl acetate was dried and evaporated to dryness and the residue was purified by flash chromatography on silica (Merck, ART 9385) using increasingly polar mixtures of ethyl acetate and hexane as eluent and the product was recrystallized from carbon tetrachloride to give (2RS)-2-(Z-Leu-Leu-NH)-2-methoxyacetonitrile (190 mg). M/z 447 $(MH)^+$; NMR 0.75-0.95 (m, 12H), 1.25-1.75 (m, 6H), 3.3 (s, 3H), 4.05 (m, 1H), 4.2-4.4 (m, 1H),5.0 (s, 2H), 5.95 (m, 1H), 7.3-7.4 (m, 6H), 7.95 (m, 1H), 9.5-9.7 (m, 1H).

Example 31-33:

The following compounds were prepared by a similar process to that described in Example 30:



Example	R^1	AA^1	AA^2	R^2	Mp (°C)	MH^+
31 [4]	Z	Leu	Leu	i-PrO		475
32 [4,5]	Z	Leu	Leu	2-propynyloxy		471
33 [4,5]	Z	Leu	Leu	Pyrazol-1-yl	183-184	483

Example 34

(2RS)-2-(Z-Leu-Phe-NH)-2-(pyrazol-1-yl)acetonitrile

A mixture of (2RS)-2-(Z-Leu-Phe-NH)-2-(2-propylthio)acetonitrile (105 mg), yellow mercuric oxide (100 mg), pyrazole and tetrahydrofuran (5 ml) was stirred at room temperature for 18 hours. The mixture was filtered and the filtrate was dried and evaporated to dryness. The residue was purified by flash chromatography on silica (Merck, ART 9385) using increasingly

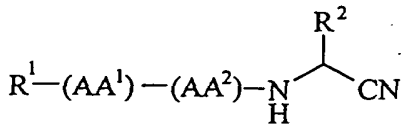
[4] mixture of epimers at aminoacetonitrile (NB varying ratios of isomers)

[5] 5 equivalents of alcohol/pyrazole in tetrahydrofuran solution and 18 hours reaction time.

polar mixtures of ethyl acetate and hexane as eluent and the product was recrystallized from a mixture of dichloromethane and hexane to give (2RS)-2-(Z-Leu-Phe-NH)-2-(pyrazol-1-yl)acetonitrile (70 mg). Mp 189-192 °C; m/z 517 $(MH)^+$; NMR 0.75-0.95 (m, 6H), 1.2-1.7 (m, 3H), 2.7-3.1 (m, 2H), 4.0 (m, 1H), 4.6 (m, 1H),5.05 (s, 2H), 6.35 (m, 1H), 7.0-7.5 (m, 12H), 7.65 (m, 1H), 7.7-7.85 (m, 1H), 7.95-8.1 (m, 1H), 10.05-10.25 (m, 1H).

Examples 35-38:

The following compounds were prepared by a similar process to that described in Example 34:



Example	R^1	AA^1	AA^2	R^2	MH^+
35	Z	Leu	Leu	imidazol-1-yl	483
36	Z	Leu	Leu	1,2,4-triazol-1-yl	484
37	Z	Leu	Leu	3,5-dimethyl-pyrazol-1-yl	511
38	Z	Leu	Leu	4-bromo-3,5-dimethyl-pyrazol-1-yl	589

Example 39

2-(Z-Leu-Cha-NH)acetonitrile

A mixture of Z-Leu-Cha-OH (0.95 g), aminoacetonitrile hydrochloride (0.23 g), 1-hydroxybenzotriazole (0.46 g), 1-dimethylaminopropyl-3-ethylcarbodiimide (0.5 g), *N,N*-dimethylformamide (6 ml) and N-methylmorpholine (0.75 ml) was stirred at room temperature for 18 hours. The mixture was diluted with ethyl acetate, and the solution was washed successively with 0.5 M hydrochloric acid, 1M sodium hydroxide and brine and then dried and evaporated to dryness. The residue was purified by flash chromatography on silica (Merck, ART 9385) using a 49:1 v:v mixture of dichloromethane and methanol as eluent and fractions containing the required product were collected, dried and evaporated to dryness. The residue was

triturated with ether and the insoluble white solid collected to give 2-(Z-Leu-Cha-NH)acetonitrile (0.46 g). M/z 493 $(MH)^+$; NMR 0.75-0.95 (m, 6H), 1.0-1.8 (m, 14H), 4.0-4.2 (m, 3H), 4.35 (q, 1H), 5.05 (s, 2H), 7.25-7.5 (m, 6H), 7.95 (d, 1H), 8.6 (m, 1H).

Example 40

2-[{2-(4-Pyridyl)acetyl}-Leu-Leu]-4-methylthiobutyronitrile

A mixture of 2-(H-Leu-Leu-NH)-4-methylthiobutyronitrile (71 mg), 1-hydroxybenzotriazole (34 mg), 1-dimethylaminopropyl-3-ethylcarbodiimide (48 mg), 2-(4-pyridyl)acetic acid hydrochloride (35 mg), *N,N*-dimethylformamide (2 ml) and N-methylmorpholine (0.2 ml) was stirred at room temperature for 18 hours. The mixture was diluted with ethyl acetate, and the solution was washed successively with 1M sodium hydroxide and brine, dried and evaporated to dryness. The residue was recrystallized from ethyl acetate to give 2-[{2-(4-pyridyl)acetyl}-Leu-Leu]-4-methylthiobutyronitrile (42 mg). Mp 177-178 °C; M/z 476 $(MH)^+$; NMR 0.75-0.95 (m,12H), 1.3-1.7 (m, 6H), 1.9-2.1 (m, 5H), 2.5 (m, 2H), 3.5 (s, 2H), 4.15-4.4 (m, 2H), 4.85 (q, 1H), 7.25 (d, 2H), 8.1 (d, 1H), 8.35 (d, 1H), 8.45 (d, 2H), 8.65 (d, 1H).

Example 41

Ac-Leu-Leu-(2-furyl)acetonitrile

Trimethylsilyl iodide (0.7 ml) was added to a solution of Boc-Leu-Leu-(2-furyl)-acetonitrile (1.68 g) in chloroform (50 ml) at 0°C. The mixture was stirred at 0°C for 15 minutes and the solvent was removed under reduced pressure. The residue was dissolved in pyridine (20 ml), the solution was cooled to 0°C and acetic anhydride (20 ml) was added and the reaction mixture was stirred at ambient temperature for 14 hours. The reaction mixture was evaporated to dryness and the residue was dissolved in ethyl acetate (100 ml) and washed with 2M hydrochloric acid, brine, saturated aqueous sodium bicarbonate solution, brine and dried. The residue obtained on removal of the solvent was triturated with diethyl ether and filtered. The solid was dissolved in warm acetone, treated with activated charcoal and filtered. The solvent was removed to give Ac-Leu-Leu-(2-furyl)acetonitrile (0.64 g). Mp 235-242°C; m/z 391 $(MH)^+$; NMR 0.88 (m, 12H), 1.51, (m, 6H), 1.83 (d, 3H), 4.29 (m, 2H), 6.23 (d, 1H), 6.53 (m, 2H), 7.76 (m, 1H), 7.95 (m, 2H), 9.19 (m, 1H).

Example 42

(2S)-2-(Boc-Leu-NH)isovaleronitrile

Trifluoroacetic anhydride (0.84 g) was added dropwise to a mixture of BOC-Leu-Leu-NH_2 (1 g) and pyridine (10 ml) which was stirred under argon at -10°C. The mixture was allowed to warm to room temperature over 1hour, diluted with water and extracted with diethyl ether. The extract was washed successively with 1M hydrochloric acid and brine, dried and evaporated to dryness. The residue was recrystallized from ether/hexane to give (2S)-2-(BOC-Leu-NH)isovaleronitrile (0.66 g). Mp 117-118°C; m/z 326 $(MH)^+$.

Example 43

(2S)-2-(Boc-Leu-NH)-4-methylthiobutyronitrile

The process described in Example 42 was repeated using BOC-Leu-Met-NH_2 instead of BOC-Leu-Leu-NH_2 to give (2S)-2-(BOC-Leu-NH)-4-methylthiobutyronitrile. Mp 69-71°C; m/z 344 $(MH)^+$.

Example 44

(2S)-2-[(N-4-chlorobenzyl-Leu)-Leu-NH]-4-methylthiobutyronitrile

A mixture of (2S)-2-[H-Leu-Leu-NH]-4-methylthiobutyronitrile (90mg), ethanol (5ml) and 4-chlorobenzaldehyde was stirred at ambient temperature for 1 hour. Acetic acid (0.05ml) was added followed by sodium cyanoborohydride (50mg) and stirring was continued for a further 3 hours. Acetic acid (0.2ml) was added and the mixture was left at room temperature for 16 hours. The mixture was diluted with water and basified with sodium hydrogen carbonate then extracted with ethyl acetate The extract was dried and evaporated to dryness and the residue was purified by flash chromatography on silica (Merck, ART 9385) using increasingly polar mixtures of ethyl acetate and hexane as eluent to give (2S)-2-[(N-4-chlorobenzyl-Leu)-Leu-NH]-4-methylthiobutyronitrile as a gum (102mg). The gum was dissolved in ethyl acetate and the solution was acidified with ethereal HCl. The mixture was evaporated to dryness and the residue was triturated with ethyl acetate and the insoluble solid collected to give (2S)-2-[(N-4-chlorobenzyl-Leu)-Leu-NH]-4-methylthiobutyronitrile hydrochloride (75mg). Mp 146-147°C;

NMR 0.9 (m, 12H), 1.4-1.8 (m, 6H), 2.0-2.2 (m, 5H), 2.55(m, 2H), 3.6 (m, 1H),3.7-4.2 (m, 2H), 4.35 (m, 1H), 4.85 (m, 1H), 7.5(m, 4H), 8.9-9.1 (m, 2H), 9.45 (m, 1H), 9.7 (m, 1H); m/z 481 $(MH)^+$.

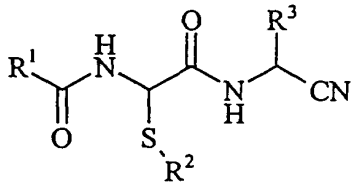
Example 45

2-[2-benzyloxycarbonylamino-2-phenylthioacetamido]-2-(2-thienyl)-acetonitrile

A mixture of 2-benzyloxycarbonylamino-2-phenylthioacetic acid (160mg), 2-amino-2-(2-thienyl)acetonitrile hydrochloride (88 mg), hydroxybenzotriazole (75 mg), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (105 mg) and 4-methylmorpholine (0.15 ml) in *N,N*-dimethylformamide (3 ml) was stirred at ambient temperature for 48 hours. The solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate (100 ml) and water (100 ml). The ethyl acetate layer was separated and washed with 2M hydrochloric acid, brine, saturated aqueous sodium bicarbonate and dried and evaporated to dryness. The residue was purified by flash chromatography on silica (Merck, ART 9385) using increasingly polar mixtures of ethyl acetate and hexane as eluent followed by recrystallisation from a mixture of ethyl acetate and hexane to give 2-(2-benzyloxycarbonylamino-2-phenylthioacetamido)-2-(2-thienyl)-acetonitrile (4.53 g). M/z 438 $(MH)^+$; NMR ($CDCl_3$) 5.1 (m, 2H), 5.5 (m, 1H), 5.85 (m, 1H), 6.2 (m, 1H), 6.8-7.1 (m, 2H),7.2-7.55 (m, 12H).

Example 46 - 55

The following examples were prepared by a process similar to that described in Example 45:



Example	R^1	R^2	R^3	Mp (°C)	MH^+
46	benzyloxy	2-propyl	H	-	322

47	benzyloxy	phenyl	H	-	356
48	benzyl	phenyl	H	-	340
49	2,4,6-trichloro phenoxymethyl	phenyl	H	153-154	458
50	naphth-2-yl	phenyl	H	151-153	376
51	2,4-dichlorophenyl	phenyl	H	171-172	394
52	naphth-2-yl	naphth-1-yl	H	190-192	426
53	naphth-2-yl	naphth-2-yl	H	186-188	426
54	phenylsulphonyl methyl	phenyl	H	189-190	404
55	benzyloxy	m-tolyl	thiophen-2-yl	118-121	452

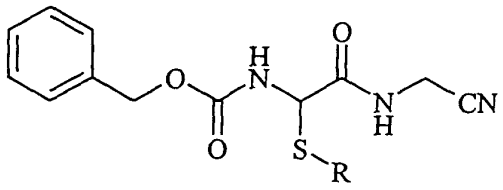
Example 56

2-[2-benzyloxycarbonylamino-2-(4-chlorophenylthio)acetamido]-2-(2-thienyl)-acetonitrile

A mixture of 2-[2-benzyloxycarbonylamino-2-(hydroxy)acetamido]-2-(2-thienyl)-acetonitrile (132 mg), 1,2-dichloroethane (5 ml), 2-naphthalenesulphonic acid (5 mg) and 4-chlorothiophenol (1.65 g) was stirred at reflux for 2 hours then cooled to room temperature and evaporated to dryness. The residue was dissolved in ethyl acetate and the solution was washed successively with 1M NaOH and brine, dried and evaporated to dryness. The residue was recrystallized from ethyl acetate and hexane to give 2-[2-benzyloxycarbonylamino-2-(4-chlorophenylthio)acetamido]-2-(2-thienyl)-acetonitrile (80 mg). Mp 165-166°C; m/z 390 $(MH)^+$; NMR 4.2 (d, 2H), 5.0 (q, 2 1H), 5.7 (d, 1H), 7.2-7.55 (m, 9H), 8.2 (d, 1H), 9.0 (t, 1H).

Examples 57-8

The following examples were prepared by a process similar to that described in Example 56:



Example	R	Mp (°C)	MH^+
57	3-chlorophenyl	149-150	390
58	benzyl	139-140	370

Preparation of Starting Materials

The starting materials for the Examples above are either commercially available or are readily prepared by standard methods from known materials. For example the following reactions (Methods A-H) are illustrations but not limitations of the preparation of some of the starting materials used in the above reactions.

Method A

2-(2-Furyl)-2-aminoacetonitrile

Ammonium chloride (25 g) was added to a solution of 2-furfuraldehyde (25 g) in diethyl ether (250 ml). A solution of sodium cyanide (17 g) in water (80 ml) was added over 20 minutes. The reaction mixture was stirred at ambient temperature for 14 hours , the aqueous layer was removed and the organic layer was washed twice with saturated aqueous sodium hydrogen carbonate solution (100 ml each time), dried and evaporated to dryness. The residue was dissolved in diethyl ether (250 ml) and cooled to 0 °C. Hydrogen chloride gas was bubbled through the solution keeping the temperature below 10 °C. 2-(2-Furyl)-2-aminoacetonitrile hydrochloride was filtered and dried, yield 33 g. ^{1}H NMR 6.19 (s, 1H), 6.56 (m, 1H), 6.78 (d, 1H), 7.83 (m, 1H), 9.83 (broad s, 2H).

Method A1

Following the method outlined in Method A and using the appropriate aldehyde there was prepared:

A1 2-(2-thienyl)-2-aminoacetonitrile hydrochloride

Method B

Z-Leu-Leu-Phe-NH_2

A mixture of H-Leu-Phe-NH_2 hydrochloride (0.8 g), Z-Leu-OH (0.72 g), *N,N*-dimethylformamide (10 ml), 1-hydroxybenzotriazole (0.41 g), 1-dimethylaminopropyl-3-ethylcarbodiimide hydrochloride (0.57 g) and N-methylmorpholine (1 ml) was stirred at room temperature for 18 hours. The mixture was evaporated to dryness and the residue stirred with water and ethyl acetate. The insoluble solid was collected to give Z-Leu-Leu-Phe-NH_2 (0.67 g). M/z 525 $(MH)^+$.

Method B1

Following the method outlined in Method C and using the appropriate protected starting materials there was prepared:

Method	Product	Starting Materials	$(MH)^+$
B1	Z-Phe-Leu-Met-NH_2	H-Leu-Met-NH_2 and Z-Phe-OH	543

Method C

Boc-Leu-Met-NH_2

A mixture of Leu-Met-NH_2 hydrochloride (0.45 g), BOC-Leu-OH (0.35 g), *N,N*-dimethylformamide (5 ml), 1-hydroxybenzotriazole (0.23 g), dicyclohexylcarbodiimide (0.35 g) and N-methylmorpholine (0.2 ml) was stirred at room temperature for 18hours. The mixture was filtered and the filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate. The solution was washed successively with 0.5 M hydrochloric acid, 1M sodium hydroxide and brine, dried and evaporated to dryness. The residue was recrystallized from a mixture of ethyl acetate and hexane to give BOC-Leu-Met-NH_2 (0.45 g). Mp145-147°C; m/z 475 $(MH)^+$.

Methods C1-2

Following the method outlined in Method C and using the appropriate protected amino acid there was prepared:

Method	Product	Amino Acid	$(MH)^+$
C1	Z-Leu-Leu-Met-NH_2	Z-Leu-OH	509
C2	[(2RS)-N-benzoyl-3-(4-pyridyl)alaninyl]-Leu-Met-NH_2	(2RS)-N-benzoyl-3-(4-pyridyl)alanine	509

Method D

2-(2-propylthio)-2-(Z-Leu-Phe-NH)acetic acid

A mixture of Z-Leu-Phe-NH_2 (3 g), glyoxylic acid monohydrate (0.83 g) and dioxan (20 ml) was stirred at reflux for 18 hours, cooled and evaporated to dryness. The residue was treated with dichloroethane (20 ml), 2-propane thiol (2.25 g) and naphthalene-2-sulphonic acid (50 mg) and the mixture was stirred at 60°C for 4 hours and then evaporated to dryness. The residue was stirred with ether and the insoluble white solid was collected to give 2-(2-propylthio)-2-(Z-Leu-Phe-NH)acetic acid (2.1 g). Mp 156-158°C; m/z 544 $(MH)^+$.

Methods D1-2

Following the method outlined in Method D and using the appropriate dipeptide there was prepared:

Method	Product	Dipeptide
D1	2-(2-propylthio)-2-(Z-Leu-Leu-NH)acetic acid	Z-Leu-Leu-NH_2
D2	2-(2-propylthio)-2-(Z-Phe-Leu-NH)acetic acid	Z-Phe-Leu-NH_2

Method E

2-(2-propylthio)-2-(Z-Leu-Phe-NH)acetamide

A mixture of 2-(2-propylthio)-2-(Z-Leu-Phe-NH)acetic acid (2 g), ammonium chloride (0.54 g), *N,N*-dimethylformamide (15 ml), 1-hydroxybenzotriazole (0.67 g), 1-dimethylaminopropyl-3-ethylcarbodiimide (0.95 g) and N-methylmorpholine (2 ml) was stirred at room temperature for 18 hours. The mixture was evaporated to dryness and the residue taken up in ethyl acetate. The solution was washed successively with 0.5 M hydrochloric acid, 1 M sodium hydroxide and brine. The solid which separated from the ethyl acetate phase was collected to give 2-(2-propylthio)-2-(Z-Leu-Phe-NH)acetamide, (1 g). Mp198-201°C; m/z 543 $(MH)^+$.

Methods E1-2

Following the method outlined in Method E and using the appropriate acetic acid there was prepared:

Method	Product	Acetic Acid	$(MH)^+$
E1	2-(2-propylthio)-2-(Z-Leu-Leu-NH)acetamide	2-(2-propylthio)-2-(Z-Leu-Leu-NH)acetic acid	509
E2	2-(2-propylthio)-2-(Z-Phe-Leu-NH)acetamide	2-(2-propylthio)-2-(Z-Phe-Leu-NH)acetic acid	543

Method F

Z-Leu-Cha-OH

A mixture of Z-Leu-OH (2.77 g), (S)-3-cyclohexylalanine methyl ester hydrochloride (1.92 g), 1-hydroxybenzotriazole (1.76 g), 1-dimethylaminopropyl-3-ethylcarbodiimide (1.92 g), *N,N*-dimethylformamide (10 ml) and N-methylmorpholine (3 ml) was stirred at room temperature for 18 hours. The mixture was diluted with ethyl acetate, and the solution was washed successively with 0.5 M hydrochloric acid, 1M sodium hydroxide and brine, then dried and evaporated to dryness. The residue was stirred in a mixture of tetrahydrofuran (15 ml) and 1M sodium hydroxide for 1hour at room temperature. The mixture was acidified with 2M

hydrochloric acid and extracted with ethyl acetate. The ethyl acetate layer was washed with brine, dried and evaporated to dryness to give Z-Leu-Cha-OH. M/z 419 $(MH)^+$.

Method G

(2S)-2-(H-Leu-Leu-NH)-4-methylthiobutyronitrile

Iodotrimethylsilane (0.6 ml) was added dropwise to a stirred solution of (2S)-2-(BOC-Leu-Leu-NH)-4-methylthiobutyronitrile (0.65 g) in dichloromethane (10 ml) the mixture was stirred at room temperature for 1 hour and then evaporated to dryness. The residue was redissolved in dichloromethane and the solution was treated with methanol (1 ml) and stirred until effervescence ceased. The solution was washed with aqueous sodium bicarbonate solution, dried and evaporated to dryness to give (2S)-2-(H-Leu-Leu-NH)-4-methylthiobutyronitrile as a gum (0.45 g). M/z 357 $(MH)^+$.

Method H

Boc-Leu-Leu-NH_2

A mixture of BOC-Leu-OH (6.93 g), Leu-OMe hydrochloride (5.45 g), 1-hydroxybenzotriazole (4.73 g), 1-dimethylaminopropyl-3-ethylcarbodiimide (6.7 g), *N,N*-dimethylformamide (50 ml) and N-methylmorpholine (10 ml) was stirred at room temperature for 18 hours. The mixture was evaporated to dryness and the residue was partitioned between water and ethyl acetate. The organic phase was washed successively with 0.5M hydrochloric acid, 1M sodium hydroxide and brine and then dried and evaporated to dryness. The residue was triturated with a mixture of ether and hexane to give Boc-Leu-Leu-OMe (11 g).

A mixture of a portion of the above ester (4 g), methanol (30 ml) and concentrated aqueous ammonia (50 ml) was stirred at room temperature for 72 hours. The mixture was diluted with water and extracted with ethyl acetate. The extract was washed with brine, dried and evaporated to dryness to give Boc-Leu-Leu-NH_2 (3.3 g). M/z 344.5 $(MH)^+$.

Method I

(2S)-2-[(N-phenylacetyl-N-methyl-Leu)-Leu-Met-NH_2

Phenylacetyl chloride (0.57 g) was added, dropwise to a stirred, ice cooled solution of N-methyl-Leu (0.53 g) in 2M sodium hydroxide (4 ml) and the mixture was stirred at ambient temperature for 3 hours. The mixture was washed with ether and the aqueous phase was acidified with 2M hydrochloric acid and the precipitate collected to give N-phenylacetyl-N-methyl-Leu-OH (0.33 g). Mp 146-147°C; m/z 264 $(MH)^+$.

This was then coupled with H-Leu-Met-NH_2 according to method C to give (2S)-2-[(N-phenylacetyl-N-methyl-Leu)-Leu-Met-NH_2. M/z 507 $(MH)^+$.

Method I1

(2S)-2-[(N-benzoyl-N-methyl-Leu)-Leu-Met-NH_2 [m/z 493 $(MH)^+$] was also obtained by the process described in Method H.

Method J

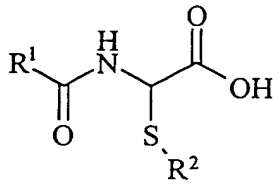
2-benzyloxycarbonylamino-2-phenylthioacetic acid

A mixture of benzyl carbamate (15.1 g), ether (100ml) and glyoxylic acid monohydrate (10.1 g) was stirred at room temperature for 16 hours. The thick suspension was filtered and the residue was washed with a mixture of ether and hexane to give 2-benzyloxycarbonylamino glycollic acid (17 g), which was used without further purification.

A mixture of 2-benzyloxycarbonylamino glycollic acid (2.25 g), 1,2-dichloroethane (50ml) and thiophenol (1.65 g) was stirred at reflux for 2 hours then cooled to room temperature. The mixture was extracted twice with aqueous sodium hydrogen carbonate and the combined extracts were washed with ether then acidified with 2M hydrochloric acid. The mixture was extracted with ether and the extract was dried and evaporated to dryness to give 2-benzyloxycarbonylamino-2-phenylthioacetic acid as a white solid (2.45 g). M/z 318 $(MH)^+$.

Method J 1 - 4

Following the method outlined in Method J and using the appropriate amide instead of benzyl carbamate in the first stage and the appropriate thiol in the second stage there was prepared:



Method	R^1	R^2	MH^+
J1	benzyloxy	2-propyl	322
J2	2,4,6-trichlorophenoxymethyl	phenyl	420
J3	2,4-dichlorophenyl	phenyl	356
J4	benzyloxy	m-tolyl	332

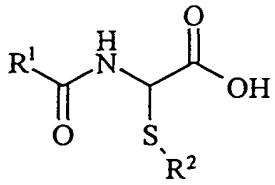
Method K

2-phenylacetamido-2-phenylthioacetic acid

A mixture of phenylacetamide (2.7 g), glyoxylic acid monohydrate (2.02 g), 1,2-dichloroethane (100 ml), thiophenol (3.3 g) and 2-naphthalenesulphonic acid (100 mg) was stirred at reflux for 16 hours. The mixture was cooled and extracted twice with aqueous sodium hydrogen carbonate and the combined extracts were washed with ether, acidified with 2M hydrochloric acid and extracted with ether. The ether extract was dried and evaporated to dryness to give 2-phenylacetamido-2-phenylthioacetic acid. M/z 302 (MH+).

Method K 1 - 4

Following the method outlined in Method K and using the appropriate starting materials there was prepared:



Method	R^1	R^2	MH^+
K1	naphth-2-yl	phenyl	338
K2	naphth-2-yl	naphth-1-yl	388
K3	naphth-2-yl	naphth-2-yl	388
K4	phenylsulphonylmethyl	phenyl	366

Method L

2-[2-benzyloxycarbonylamino-2-(hydroxy)acetamido]-2-(2-thienyl)-acetonitrile

A mixture of 2-benzyloxycarbonyamino glycollic acid (1.125 g), 2-aminoacetonitrile hydrochloride (0.7 g), hydroxybenzotriazole (0.75 g), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.05 g) and 4-methylmorpholine (3 ml) in *N,N*-dimethylformamide (20 ml) was stirred at ambient temperature for 18 hours. The solvent was removed under reduced pressure and the residue was stirred with a mixture of ethyl acetate (100 ml) and 1M Hydrochloric acid and the insoluble solid collected to give 2-[2-benzyloxycarbonylamino-2-(hydroxy)acetamido]-2-(2-thienyl)-acetonitrile. Mp 146-148°C; m/z 264 $(MH)^+$.

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